Plant Physiol. (1973) 51, 448-453

Multiple Forms of Plant Phosphoenolpyruvate Carboxylase Associated with Different Metabolic Pathways[1]

Received for publication August 8, 1972

IRWIN P. TING AND C. B. OSMOND
Department of Biology, University of California, Riverside, California 92502 and Department of Environmental Biology, Research School of Biological Sciences, A.N.U., Canberra, 2601, Australia

ABSTRACT

The physical and kinetic properties of multiple forms of phosphoenolpyruvate carboxylase were studied in leaves of C_4 and C_3 species, their F_1 and F_3 hybrids, in greening maize leaves, in Crassulacean acid metabolism plants, and in nongreen root tissues. Four different forms are suggested: a C_4 photosynthetic phosphoenolpyruvate carboxylase with high Km for phosphoenolpyruvate (~0.59 mM), Km Mg (~0.5 mM), and V_{max} (~29 micromoles per minute per milligram of chlorophyll); a C_3 photosynthetic phosphoenolpyruvate carboxylase with low Km for phosphoenolpyruvate (~0.14 mM), Km for Mg (~0.097 mM), and V_{max} (1.5); a Crassulacean acid metabolism type with low Km for phosphoenolpyruvate (0.14 mM), and high V_{max} (14 micromoles per minute per milligram of chlorophyll); and a nongreen or nonautotrophic type with low Km for phosphoenolpyruvate, Km for Mg, and low V_{max}. In closely related species or within species, the types can be differentiated by anion exchange column chromatography. Each of the four forms is associated with a different metabolic pathway: the phosphoenolpyruvate carboxylase of C_4 species for malate generation as a photosynthetic intermediate, the phosphoenolpyruvate carboxylase of C_3 species in malate generation as a photosynthetic product, the phosphoenolpyruvate carboxylase of Crassulacean acid metabolism species in malate generation as a CO_2 donor for photosynthesis during the subsequent light period, and a nongreen or root type producing malate for ionic balance and reduced nicotinamide adenine dinucleotide phosphate generation. The data in this paper in conjunction with published information support the notion of different molecular forms of a protein functioning in different metabolic pathways which have common enzymic steps.

In a previous report, we showed that the PEP[2] carboxylases [orthophosphate:oxaloacetate carboxylase (phosphorylating) EC 4.1.1.31] from leaves of C_3 and C_4 plants differed in several important kinetic parameters as well as chromatographic behavior on anion exchange columns (17). It was emphasized that, although both enzymes function in the generation of malate and aspartate during photosynthesis, the metabolic role of the C_4 acids in the two photosynthetic systems is quite different. In leaves of C_4 plants malate and aspartate are photosynthetic intermediates whereas these compounds are products of photosynthesis in leaves of C_3 plants (3, 8). Our unpublished data indicate that in C_3 *Atriplex patula* malate accumulates at the expense of phosphoglycerate in pulse chase experiments (Osmond, unpublished). These experiments support the general notion that physically different classes of the same enzyme can be associated with different metabolic pathways involving a common enzymic step.

In this paper we further extend the comparison of PEP carboxylase alloenzymes[3] in C_3 and C_4 plants showing the inheritance of both enzymes in hybrids between C_3 and C_4 *Atriplex* spp. and the changes in characteristics of PEP carboxylase during greening of etiolated seedlings of *Zea mays*. The hypothesis that specific forms of PEP carboxylase are also associated with CO_2 fixation events outside C_3 and C_4 photosynthesis is confirmed by studies on the behavior of the enzyme from root tissues and from leaves of Crassulacean acid metabolism plants. These data suggest that at least four different forms of PEP carboxylase protein exist in higher plants: a C_3-photosynthetic PEP carboxylase, a C_4-photosynthetic PEP carboxylase, a CAM-PEP carboxylase, and a dark or nonautotrophic PEP carboxylase.

MATERIALS AND METHODS

Seedlings of *Atriplex spongiosa* FvM, *A. rosea* L., *A. hastata* L., *A. patula* ssp. *hastata* Hallsand Chem., and *Vicia faba* L., were grown in water culture in the glasshouse as described earlier (17). Individuals of the F_3 hybrids of the cross *A. rosea* (♀) and *A. patula* ssp. *hastata* (♂) were also grown in water culture from seed collected from F_2 7735-5. These have been designated series 8012 here, distinguishing them from the F_1 hybrid 8001-2 and other F_3 hybrids studied earlier (9). Seedlings of *Amaranthus edulis, Panicum miliaceum* L., *Sorghum bicolor* L., *Saccharum officinarum* L., and the CAM plants *Kalanchoe diagremontiana* Hamet et Perrier, *Bryophyllum pinnatum* (Lamk.) Oken, *B. tubiflorum* Harv., *Opuntia inermis* Haw, and *Sedum praeltum* L. were grown in soil in the greenhouse or outdoors. Seedlings of *Zea mays* L., variety NES 1002, were grown in coarse sandy soil in the greenhouse for 2 to 3 weeks or were grown in total darkness for 10 days. Mature leaves of the dicotyledons and the third and subsequent leaves

[1] This work was supported in part by National Science Foundation Grant GB 25878 and an Australian National University Visiting Fellowship to Irwin P. Ting.

[2] Abbreviations: CAM: Crassulacean acid metabolism; PEP: phosphoenolpyruvate; DIECA: diethyldithiocarbamate; PVP: polyvinylpyrollidone; DTT: dithiotreitol; DEAE; diethylaminoethyl.

[3] Alloenzyme, referring to different forms of a particular enzyme from different species, should not be used interchangeably with isoenzyme, referring to different forms of a particular enzyme within a species, variety, etc (2). In this paper we adopt the more inclusive term "multiple forms" to embrace both situations.

of the monocotyledons were harvested. Entire root systems of water culture-grown plants or roots of 3-day dark-germinated seedlings of *Z. mays* were harvested.

Extraction and Chromatography of PEP Carboxylases. Approximately 20 g of leaf or root material were chopped finely with a blade and extracted in 50 ml of 50 mM Bicine buffer, pH 7.8, containing 2 mM DTT, 2 mM EDTA, and 1% PVP (molecular weight 360,000) for two 10-sec intervals at full speed in a Sorvall Omnimixer. When green grass leaves were used, 10 mM sodium DIECA was included in the buffer. The remainder was processed as described previously (17) and the 40 to 55% saturated ammonium sulfate precipitate was chromatographed on a 1.5- × 15-cm DEAE-cellulose column with a 0.02 to 0.2 M linear phosphate gradient as described elsewhere (12). After 30- × 7.5-ml fractions had been collected, the phosphate gradient was sometimes replaced by 0.2 M phosphate to elute slowly moving components. An aliquot of the fresh homogenate was taken for chlorophyll estimation, and the rest was centrifuged for 30 min at 28,000*g*. A further aliquot was taken for estimation of maximal velocity of PEP carboxylase activity using 0.1 to 2.0 mM PEP. Homogenates from CAM plants were desalted by gel filtration through a 1- × 10-cm Sephadex G-25 column. PEP carboxylase activity was assayed by the coupled spectrophotometric procedure using an excess of pig heart crystalline malate dehydrogenase (15). The enzyme was recovered from active column fractions, precipitated with 60% ammonium sulfate, and prepared for kinetic analysis.

Greening Experiments. Etiolated seedlings of *Z. mays* were exposed to 65,000 ergs cm^{-2} sec^{-1} total radiation from a single 400 W Philips HPLR lamp. At intervals, 5 g of leaf material were extracted in 20 ml of the above buffer by grinding for 30 sec at full speed in a Sorvall Omnimixer. The fresh homogenate was filtered through Miracloth and centrifuged for 30 min at 28,000 *g*. The PEP carboxylase activity in this extract was assayed as described above, using 0.1 to 2.0 mM PEP. Another sample of 1 or 2 g of leaf material was exhaustively extracted with cold 80% acetone, and the total chlorophyll content was calculated from the absorbance values at 645 and 663 nm (6).

RESULTS

Photosynthetic PEP Carboxylases in Hybrids of C_4 and C_3 Species of *Atriplex*. In an earlier study, the alloenzymes of PEP carboxylase from leaves of *A. spongiosa* (C_4) and *A. hastata* (C_3) were examined in detail (17). The alloenzymes were distinguished on the basis of DEAE-cellulose chromatography and kinetic characteristics. The kinetic characteristics of the alloenzymes were common to a large number of C_4 and C_3 species. In this report, another pair of *Atriplex* species has been examined in detail, and Figure 1 shows that the elution profiles for PEP carboxylase from *A. rosea* (C_4) and *A. patula* spp. *hastata* (C_3) exactly reproduce those found earlier for *A. spongiosa* and *A. hastata* (17). The K*m* PEP values of 0.62 mM and 0.11 mM for *A. rosea* and *A. patula* ssp. *hastata*, respectively, are very close to those recorded for *A. spongiosa* (0.49 mM) and *A. hastata* (0.08 mM).

A. rosea (♀) *A. patula* (♂) crosses have been prepared which yield a vigorous F_1 hybrid with $2n = 18$ (identical to the parents) and F_2 hybrids exhibiting a wide range of vigor and ploidy (4). None of these hybrids has the capacity for normal C_4 photosynthesis (4, 5) although many contain quite high levels of PEP carboxylase and synthesize large proportions of malate (5). Figure 1 shows that the intermediate PEP carboxylase activity of the F_1 hybrid 8001/2 is contained in two peaks of activity which elute from DEAE-cellulose in positions corresponding to those of the C_4 and C_3 parents. The

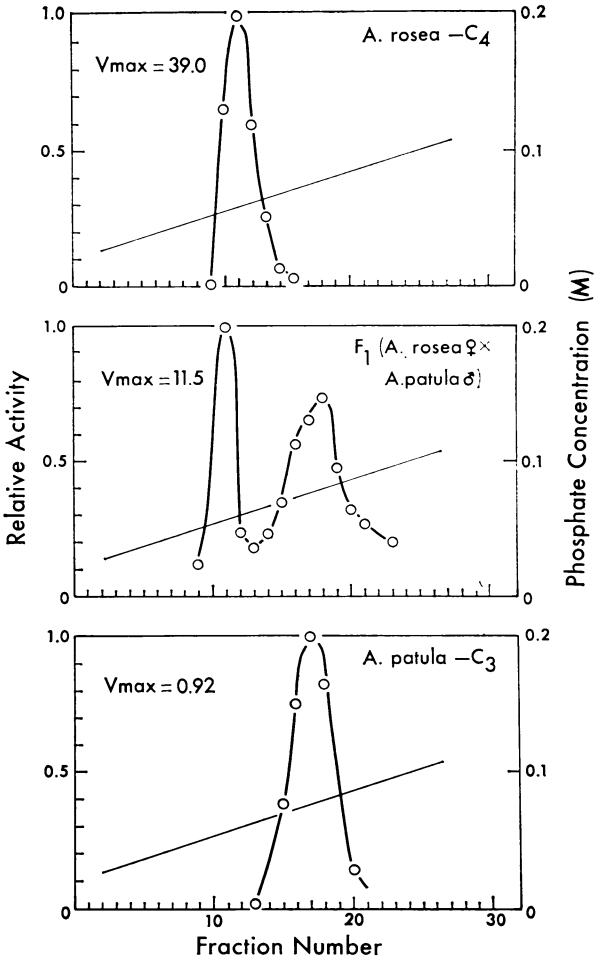

FIG. 1. The elution profiles of PEP carboxylase activity in the 40 to 55% saturated ammonium sulfate fraction of extracts of *Atriplex* leaves on DEAE-cellulose using a phosphate gradient. Data shown are from *A. rosea*, *A. patula*, and their F_1 hybrid. V_{max} = μmoles/min·mg chl.

peaks were recovered by ammonium sulfate precipitation and, after dialysis against 50 mM Bicine buffer, pH 7.8, were examined kinetically. Peak 1 corresponded chromatographically and kinetically (K*m* PEP = 0.67 mM) to that of PEP carboxylase from the C_4 parent, *A. rosea*, and peak II corresponded chromatographically and kinetically (K*m* PEP = 0.15 mM) to that of *A. patula* ssp. *hastata*, the C_3 parent. Integration of the area under the curve for the F_1 hybrid shows that 33% of the activity could be ascribed to the C_4 form of the enzyme and 67% to the C_3 form. Accurate partitioning of the relative contributions of both forms is difficult because of the possibility of differential loss in activity during DEAE-cellulose chromatography.

In a random survey of six F_2 hybrids, all were found to have both forms of PEP carboxylase corresponding to those of the C_3 and C_4 parents (Fig. 2). The maximal velocity of the PEP carboxylase in extracts of hybrids ranged from 1.97 to 10.4 μmoles/min·mg chl. As the total PEP carboxylase activity in the leaves increased, there was a trend toward more of the C_4 form relative to the C_3 form (Table I). In the case of the lowest activity encountered (1.97 μmoles/min·mg chl), 84% of the total activity was associated with the C_3 form of PEP carboxylase. For the highest activity measured (10.4 μmoles/min·mg chl), 73% of the activity was associated with the C_4 form of PEP carboxylase.

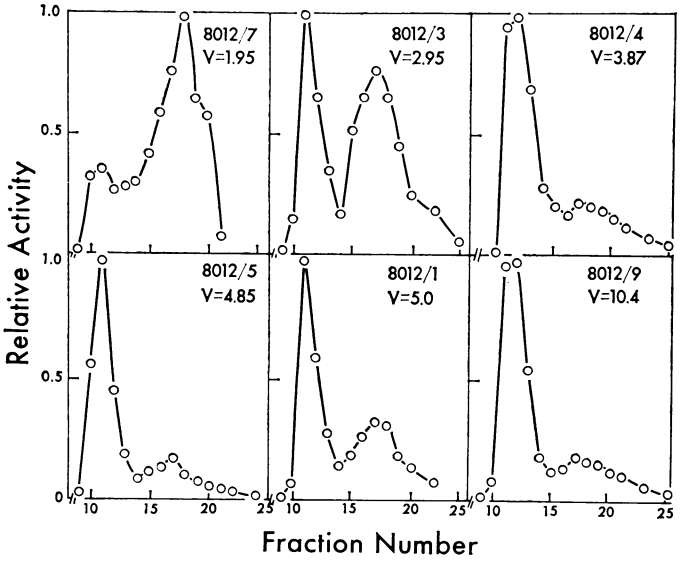

FIG. 2. The elution profiles of PEP carboxylase activity in the 40 to 55% saturated ammonium sulfate fraction of extracts of leaves of F_3 hybrids of C_3 and C_4 *Atriplex* on DEAE-cellulose using a phosphate gradient.

Table I. *Quantitative Comparison of PEP Carboxylase Activity in Atriplex F_3 Hybrids Recovered from DEAE-cellulose Columns*

Hybrid	V_{max}[1]	C_4[2]	C_3[2]	C_4/C_3
	μmoles/min·mg chl	%	%	
8012/7	1.97	16.3	83.7	0.2
8012/3	2.95	38.5	61.5	0.63
8012/4	3.87	72.1	27.9	2.58
8012/5	4.85	74.8	25.2	3.00
8012/1	5.00	55.6	44.4	1.3
8012/9	10.4	73.1	26.9	2.7

[1] V_{max} = maximal PEP carboxylase activity in fresh homogenates at saturating PEP.

[2] Percentage of recovered activity as estimated from the area of the peaks in Figure 2.

Comparison of Leaf and Root Forms of PEP Carboxylase. The two forms of PEP carboxylase found in leaves of C_3 and C_4 species of *Atriplex* show remarkably consistent chromatographic and kinetic characteristics. Some of these characteristics are shared by PEP carboxylases isolated from other dicotyledons. For example, the leaf PEP carboxylase from C_4 *Amaranthus edulis,* which has an unusually low K*m* PEP for C_4 plants (17), elutes from DEAE-cellulose in fraction 9 close to that of the C_4 *Atriplex* spp. The leaf PEP carboxylase from C_3 *Vicia faba* elutes in fraction 27, somewhat later than the C_3 *Atriplex* spp. However, in C_4 and C_3 *Atriplex* the PEP carboxylase of roots elutes from DEAE-cellulose only after the addition of 0.2 M phosphate to the column. Figure 3 shows characteristic elution profiles for leaf PEP carboxylases from *A. spongiosa* and *A. hastata,* and on the same graph are shown the elution profiles for the enzyme prepared from roots of these two species. The root enzymes from both *Atriplex* spp. show similar chromatographic and kinetic characteristics distinct from either leaf form of PEP carboxylase. The V_{max} estimates for the root PEP carboxylases of *A. spongiosa* and *A. hastata* were 0.20 and 0.18 μmole/min·g fresh weight, respectively. K*m* PEP estimates, determined with a 40 to 55% ammonium sulfate fraction, were 0.30 and 0.20 mM for *A. spongiosa* and *A. hastata.*

Further evidence for a unique root form of PEP carboxylase comes from experiments with *Z. mays.* The predominant PEP carboxylase in young roots of *Z. mays* is chromatographically and kinetically distinct from the PEP carboxylase of green or etiolated leaf tissue (Fig. 4). Most of the root enzyme elutes from DEAE-cellulose after the addition of 0.2 M phosphate and is chromatographically and kinetically similar to the root enzyme from *Atriplex* spp. (Figs. 3 and 4). The K*m* PEP estimates for green leaf, etiolated leaf, and root PEP carboxylases were 0.34, 0.19, and 0.19 mM, respectively.

There is no evidence at present for the occurrence in leaves of a form of PEP carboxylase corresponding to that of roots. When *Z. mays* leaf PEP carboxylase, for example, is eluted for 30 × 7.5 ml with the linear phosphate gradient and then eluted with 0.2 M phosphate, a slowly moving peak of PEP carboxylase activity elutes in the position of the root enzyme. However, this PEP carboxylase is kinetically identical to the principal leaf component and in all probability represents a "tail" of this component which is removed in bulk at higher ionic strength.

Multiple forms of PEP Carboxylase in Greening *Z. mays*. Figure 4 shows that the PEP carboxylase of etiolated *Z. mays* leaves elutes from DEAE-cellulose earlier than that of green *Z. mays.* Furthermore, the enzyme from green leaves has a higher K*m* PEP (0.34 mM) and V_{max} (19.2 μmoles/min·g fresh weight) than the PEP carboxylase from etiolated leaves (K*m* PEP = 0.19 mM, V_{max} = 4.9). These features were examined in more detail during a prolonged experiment in which etiolated leaves were exposed to continuous light. Figure 5 shows that there is a lag of several hours in the formation of chlorophyll and the increase in PEP carboxylase activity, and the two processes are not necessarily in phase.

A chromatographic study during the first hours of illumination suggests that the change from the form of PEP carboxylase in etiolated leaves to that in green leaves occurred prior to a measurable increase in activity (Fig. 6). The elution profiles in Figure 6 were obtained with PEP carboxylase prepared at

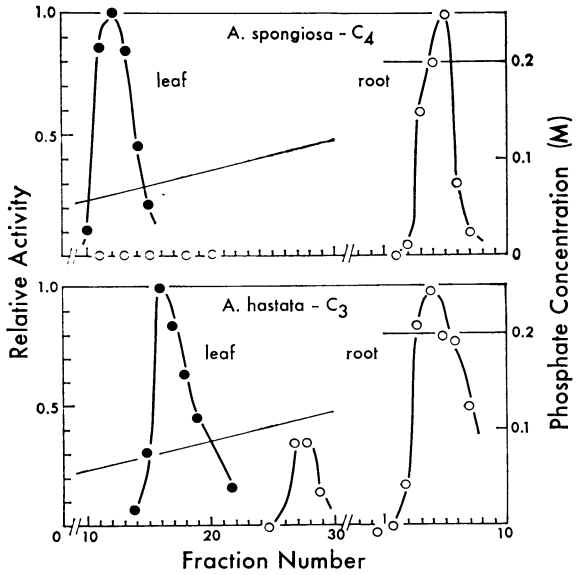

FIG. 3. The elution profiles of PEP carboxylase activity in the 40 to 55% saturated ammonium sulfate fraction of extracts of leaves and roots of *Atriplex* on DEAE-cellulose using a phosphate gradient.

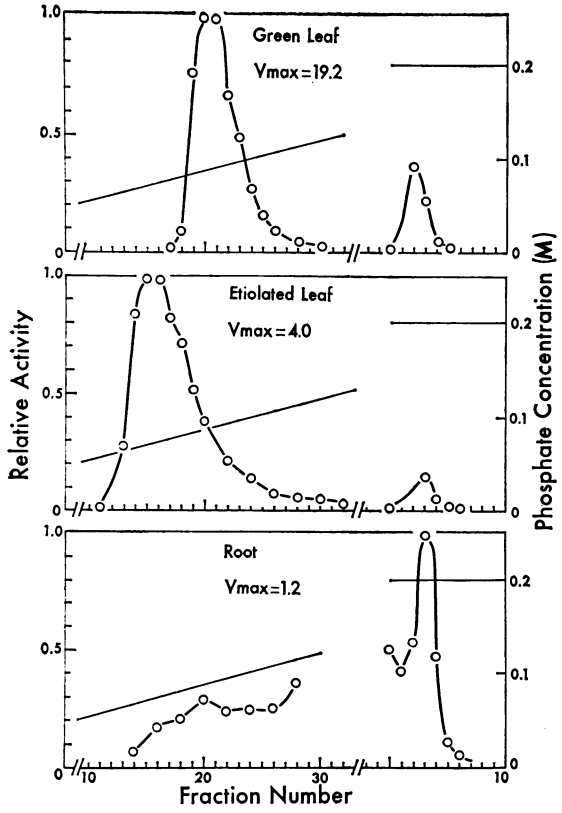

FIG. 4. The elution profiles of PEP carboxylase activity in the 40 to 55% saturated ammonium sulfate fraction of extracts of green and etiolated leaves and of roots of *Z. mays* on DEAE-cellulose using a phosphate gradient. V_{max} = μmoles/min·g fresh wt.

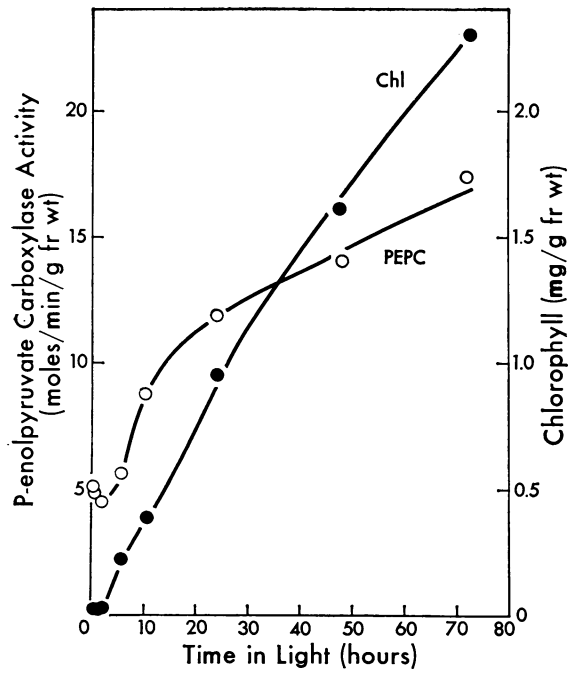

FIG. 5. Time course of greening and change in PEP carboxylase activity in etiolated leaves of *Z. mays* transferred to continuous light.

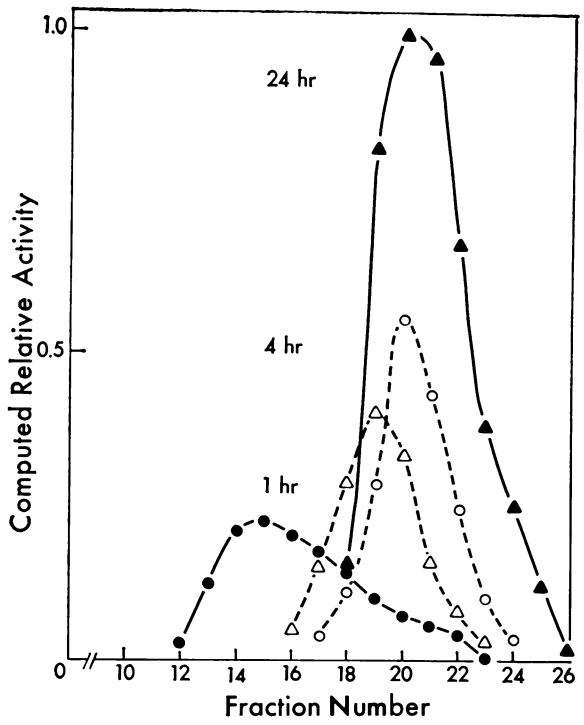

FIG. 6. Changes in elution profile and activity, illustrated by the area under the curves, of PEP carboxylase in etiolated leaves of *Z. mays* transferred to continuous light. ●: dark; △: 1 hr light; ○: 4 hr light; ▲: 24 hr light.

the indicated times after illumination, and relative activity was computed by adjusting the area under the elution curve to correspond to changes in total activity measured in Figure 5. The data show that the elution profile shifts from that of the etiolated to the green form during the first 2 to 4 hr of illumination and that the increase in activity occurs subsequent to this transformation.

There is a danger that the transformations observed during greening of *Z. mays* are artifacts of other changes such as an increased production of tannins. Tannins are a particular problem in the preparation of monocotyledon PEP carboxylase for DEAE-cellulose chromatography. Both PVP and the polyphenoloxidase inhibitor DIECA were required during extraction of PEP carboxylase from these tissues. In their absence, *Z. mays* green leaf PEP carboxylase did not elute from the column as a discrete peak. We are confident that extraction with DIECA does not introduce variable artifacts because the PEP carboxylase from leaves of the closely related C_4 *Panicum miliaceum* elutes at the same position as greened *Z. mays* (peak fraction 22). Further, the PEP carboxylase from leaves of *Sorghum bicolor* and *Saccharum officinarum* (tribe Andropogoneae) show identical elution profiles (peak fraction 15).

PEP Caboxylases from Leaves of CAM Plants. The PEP carboxylases prepared from leaves of CAM plants differ from those described above. With respect to K*m* PEP and K*m* Mg, they resemble the form of PEP carboxylase from C_3 leaves, etiolated maize, or the roots of C_3 and C_4 plants with a relatively low mean K*m* PEP of 0.19 mM (Table II). With respect to V_{max}, they differ from each of the above forms and are in the range of the average V_{max} for the PEP carboxylase in leaves of C_4 plants (17). This apparent uniformity of kinetic characteristics does not extend to the chromatographic behavior, however. Figure 7 shows the elution profiles for PEP

Table II. *Kinetic Properties of PEP Carboxylase Protein Isolated from Several CAM Plants*

CAM Species	V_{max}[1]	Km PEP
	μmoles/min·mg chl	*mM*
Kalanchoe daigremontiana	12.5	0.13
Bryophyllum tubiflorum	20.2	0.22
Bryophyllum pinnatum	4.1	0.14
Sedum praealtum	39.6	0.33
Opuntia inermis	14.9	0.12
Mean	18.3	0.19

[1] Estimated after G-25 Sephadex treatment of crude extracts.

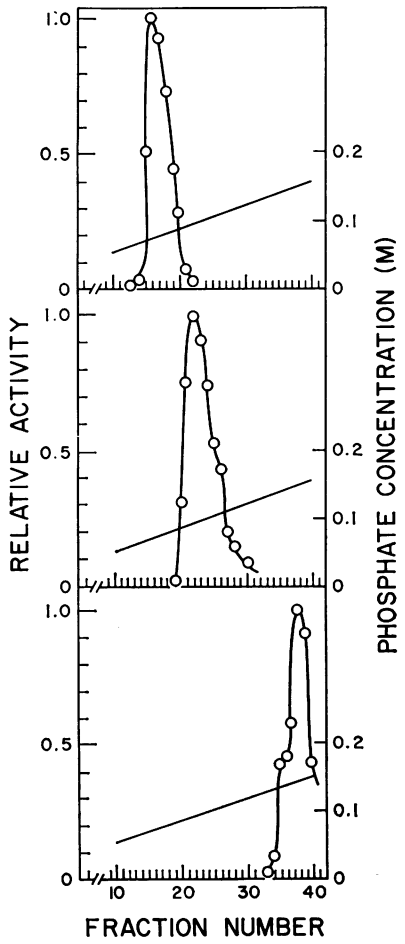

FIG. 7. The DEAE-cellulose elution profiles of PEP carboxylase activity in the 40 to 55% saturated ammonium sulfate fraction of extracts of leaves from three Crassulacean species. Top: *K. diagremontiana;* middle: *B. pinnatum;* bottom: *S. praeltum.*

carboxylase from leaves of three CAM plants. The variation in peak fraction number extends across the range found for most of the forms discussed above.

DISCUSSION

The observations in this report confirm our previous conclusions that different forms of PEP carboxylase exist in green leaves of C_4 and C_3 plants (17). Furthermore, they confirm the presence of a distinct form of PEP carboxylase in root tissues, similar to those described earlier (15). The data suggest at least four different forms of PEP carboxylase in plants which can be distinguished primarily on the basis of kinetic characteristics and, secondarily, on the basis of chromatography on DEAE-cellulose. These are: (*a*) Green leaf PEP carboxylase of C_4 plants: high K*m* PEP, K*m* Mg, and high V_{max}, chromatographically distinct from the C_3 enzyme in closely related plants such as *Atriplex* and *Amaranthus*. (*b*) Green leaf PEP carboxylase of C_3 plants: low K*m* PEP, K*m* Mg, and low V_{max}, chromatographically distinct from the root enzyme. (*c*) Root PEP carboxylase: low K*m* PEP, K*m* Mg, and low V_{max}, chromatographically similar in C_3 and C_4 plants and kinetically similar to potato tuber enzyme (14). (*d*) CAM leaf PEP carboxylase: low K*m* PEP, K*m* Mg, and high V_{max}, chromatographically variable. It should be made clear that the K*m* estimates reflect intrinsic properties of the respective proteins whereas the V_{max} estimates are a function of both turnover number of individual protein molecules and the amount of enzyme. Therefore, the differences described here may reflect both protein properties and quantities.

Each of the above generalized forms of PEP carboxylase is likely to reveal isoenzymes when chromatographed under more discriminating conditions. The shape of many elution profiles reported here is such as to suggest the presence of more than one component. However, kinetic features were remarkably constant throughout even the most "shouldered" peaks, and Mukerji and Ting found relatively little variation in K*m* PEP between three isoenzymes of C_3 cotton leaf PEP carboxylase (12).

In spite of these additional complexities, it is reasonable to consider the principal forms of higher plant PEP carboxylase in relation to participation in specific metabolic pathways. The differences between the role of PEP carboxylase during C_4 and C_3 pathways of photosynthesis have already been discussed (17). The relevance of the distinct kinetic features of these two forms of PEP carboxylase to their involvement in different metabolic pathways is not clear. In the same way, it is not clear how low K*m* PEP and low V_{max} relate to the nonautotrophic root PEP carboxylase and its role in the synthesis of malate for ionic balance and NADPH production (10, 16). In CAM plants, PEP carboxylase provides an astonishingly effective means of malate synthesis in the dark, and some of this effectiveness may be related to the combination of low K*m* PEP and high V_{max} found for this enzyme. There are conflicting reports as to the K*m* PEP for CAM PEP carboxylases, and unusually acid pH optima have been reported (11, 19).

The case for association of specific forms of PEP carboxylase with specific metabolic pathways is best supported in the C_4 and C_3 photosynthetic systems, particularly in the hybrid studies and the greening experiments. Earlier experiments with the hybrids of C_4 and C_3 *Atriplex* supported the view that unique forms of several enzymes appear to have evolved and to be necessary for the operation of the C_4 pathway of photosynthesis (9). This conclusion is supported by the more detailed comparisons made here, but it should be emphasized that the transmission of a C_4 form of PEP carboxylase to hybrid progeny does not confer a functional C_4 pathway of photosynthesis. None of the F_1, F_2, or F_3 *Atriplex* hybrids so far examined is capable of C_4 photosynthesis as judged by CO_2 compensation value, pulse-chase, and $^{13}C:^{12}C$ isotope discrimination ratio (5, 9). In an analogous way, synthesis of a new form of PEP carboxylase seems to occur during the development of the C_4 photosynthetic pathway in greening maize. The increase in PEP carboxylase activity involves synthesis of new protein (7), and the kinetic and chromatographic behavior of this protein differs from that in the etiolated tissues.

These considerations lead to a number of interesting ques-

tions not as yet answered. The central problem of identifying minority forms of PEP carboxylase was mentioned above. This is of particular interest in the intercellular localization of PEP carboxylase in C_4 plants. The bundle sheath cells of these plants are believed to contain a normal complement of carbon reduction cycle enzymes (8), presumably including a PEP carboxylase comparable to the C_3 form discussed above. In C_4 *Atriplex* spp. we have been unable to demonstrate the presence of a PEP carboxylase in bundle sheath cells with properties distinct from those of the mesophyll enzyme. Many of the F_3 hybrids show clearly defined mesophyll and bundle sheath structures and comparable quantities of C_3 and C_4 forms of PEP carboxylase. Differential grinding experiments with the hybrids containing both forms of PEP carboxylase provide no evidence that the C_3 form is preferentially associated with the bundle sheath cells. As discussed earlier, we are unable to decide if green leaves have a component PEP carboxylase resembling that of roots and functioning in nonautotrophic metabolism.

The genetic nature of the PEP carboxylases, particularly those of C_3 and C_4 photosynthesis, is important. Although the *Atriplex* hybrid experiments may indicate allelism, further genetic experiments are required. The extent of genetic determination of the differences between the forms of PEP carboxylase is interesting. For example, are the differences determined after synthesis or even during extraction? Whether very high activities of PEP carboxylase in leaves of C_4 plants and CAM plants stem from genetic control of protein structure or protein quantity remains unresolved and must await the purification of the respective proteins.

The data presented here extend the concept of organelle-specific forms of a particular enzyme involved in different metabolic pathways in different cellular compartments. Recent experiments with the NAD and NADP malate dehydrogenases of green leaf tissue show that each major subcellular compartment has a specific protein catalyzing the reduction of oxalacetate to malate (13, 18). In each case the particular forms of the protein function in relation to a specific metabolic pathway, *e.g.*, the tricarboxylic acid cycle in mitochondria and the glycolate pathway in microbodies. Multiple forms of carbon reduction cycle enzymes have also been associated with cytosol and chloroplast compartments of green leaves (1). PEP carboxylase does not show unambiguous association with organelles in plant tissues, and it is probably best regarded as a cytosol enzyme in each of the tissues discussed above. Nonetheless, the data presented here are indicative of pathway-specific forms of PEP carboxylase in tissues of higher plants. All forms initiate the synthesis of malate or aspartate. In leaves of C_4 species, a PEP carboxylase functions in the photosynthetic production of malate as an intermediate in carbon flow to carbohydrates; in C_3 species, a PEP carboxylase functions in the production of malate as a photosynthetic product; in CAM plants, a PEP carboxylase functions in the production of malate, which is subsequently used as a CO_2 donor for photosynthesis during the following light period; and in nongreen tissues, a PEP carboxylase functions in malate synthesis used in ionic balance and perhaps NADPH generation.

LITERATURE CITED

1. Anderson, L. E. and I. Pacold. 1972. Chloroplast and cytoplasmic enzymes. IV. Pea leaf fructose-1,6-diphosphate aldolase. Plant Physiol. 49: 393–397.
2. Anon. 1972. IUPAC-IUB Commission on Biochemical Nomenclature: The nomenclature of multiple forms of enzymes. Recommendations (1971). Arch. Biochem. Biophys. 258: 1–3.
3. Bassham, J. A., A. A. Benson, and M. Calvin. 1950. The path of carbon in photosynthesis. VIII. The role of malic acid. J. Biol. Chem. 185: 781–787.
4. Bjorkman, O., M. A. Nobs, and J. A. Berry. 1971. Further studies on hybrids between C_3 and C_4 species of *Atriplex*. Carnegie Inst. Wash. Yearbook 70: 507–511.
5. Bjorkman, O., M. A. Nobs, R. Pearcy, J. Boyton, and J. Berry. 1971. Characteristics of hybrids between C_3 and C_4 species of *Atriplex*. *In:* M. D. Hatch, C. B. Osmond, and R. O. Slatyer, eds., Photosynthesis and Photorespiration. Interscience, New York. pp. 105–119.
6. Bruinsma, J. 1963. The quantitative analysis of chlorophylls *a* and *b* in plant extracts. Photochem. Photobiol. 2: 241–249.
7. Graham, D., M. D. Hatch, C. R. Slack, and R. M. Smillie. 1970. Light-induced formation of enzymes of the C_4 dicarboxylic acid pathway of photosynthesis in detached leaves. Phytochemistry 9: 521–532.
8. Hatch, M. D. 1971. Mechanism and function of the C_4 pathway of photosynthesis. *In:* M. D. Hatch, C. B. Osmond, and R. O. Slatyer, eds., Photosynthesis and Photorespiration. Interscience, New York. pp. 139–152.
9. Hatch, M. D., C. B. Osmond, J. H. Troughton, and O. Bjorkman. 1972. Physiological and biochemical characteristics of C_3 and C_4 *Atriplex* species and hybrids in relation to the evolution of the C_4 pathway. Carnegie Inst. Wash. Yearbook 71: 135–141.
10. Jackson, W. A. and N. T. Coleman. 1959. Fixation of carbon dioxide by plant roots through phosphoenolpyruvate carboxylase. Plant Soil 11: 1–16.
11. Kluge, M. and C. B. Osmond. 1972. Studies on phosphoenolpyruvate carboxylase and other enzymes of Crassulacean acid metabolism of *Bryophyllum tubiflorum* and *Sedum praealtum*. Pflanzenphysiol. 66: 97–105.
12. Mukerji, S. K. and I. P. Ting. 1971. Phosphoenolpyruvate carboxylase isoenzymes: separation and properties of three forms from cotton leaf tissue. Arch. Biochem. Biophys. 142: 297–317.
13. Rocha, V. and I. P. Ting. 1971. Malate dehydrogenases of leaf tissue from *Spinacia oleracea:* properties of three isoenzymes. Arch. Biochem. Biophys. 147: 114–122.
14. Smith, T. E. 1968. Partial purification and characteristics of potato phosphoenolpyruvate carboxylase. Arch. Biochem. Biophys. 125: 178–188.
15. Ting, I. P. 1968. CO_2 metabolism in corn roots. III. Inhibition of P-enolpyruvate carboxylase by 1-malate. Plant Physiol. 43: 1919–1924.
16. Ting, I. P. 1971. Nonautotrophic CO_2 fixation and Crassulacean acid metabolism. *In:* M. D. Hatch, C. B. Osmond, and R. O. Slatyer, eds., Photosynthesis and Photorespiration. Interscience, New York, pp. 169–185.
17. Ting, I. P. and C. B. Osmond. 1973. Photosynthetic phosphoenolpyruvate carboxylases: characteristics of allozymes from leaves of C_3 and C_4 plants. Plant Physiol. 51: 439–447.
18. Ting, I. P. and V. Rocha. 1971. NADP-specific malate dehydrogenase of green spinach leaf tissue. Arch. Biochem. Biophys. 147: 156–164.
19. Walker, D. A. 1957. Physiological studies on acid metabolism. IV. Phosphoenolpyruvate carboxylase activity in extracts of Crassulacean plants. Biochem. J. 67: 73–79.